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CYTOGENETIC SCREENING OF ROMANIAN BOVINE BREEDS

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Abstract

As well as the cytogenetic investigation is a tool that could be used in the genetic improvement programmes of bovine, this paper is a review about the cytogenetic screening performed in the last 15 years on 889 cows and 317 buffaloes of different breeds, raised in Romania. The karyotype study was carried out by using the short-term blood cell culture and as a result abnormalities of chromosomal structure and number were identified in 131 cows and 51 buffaloes. The effects of the abnormal configurations were represented by reproductive activity disorders, congenital and developmental malformations, freemartinism and aneuploid-heteroploidy of sex chromosomes. The necessary prophylaxis recommendations have been released for all carriers of chromosomal abnormalities.

Key words*: buffalo, cattle, chromosomal abnormalities, congenital malformations, reproductive disorders.*

INTRODUCTION

The role of the cytogenetic screening in the genetic improvement programmes of bovine, has been demonstrated since the so-called 1/29 translocation in Swedish cattle and the effects on fertility of carriers was identified (Gustavsson & Rockborn, 1964, Gusstavsson, 1971). The presence of the chromosomal abnormalities and their relationship with the degradation of reproductive performances, congenital and developmental malformations both in cattle and buffalo (Ducos et al., 2008; Nicolae et al., 2010; Perucatti et al., 2016) is very well known and therefore has become an essential condition for improving the genetic health of farm animals. For this reason, the main objective of our cytogenetic screening, was to detect the number and frequency of the abnormal configurations and remove the carriers from the herd. It is mandatory to avoid the dissemination of abnormalities in the offspring, especially when artificial inseminations are applied. The current paper is an overview about the results of the systematic chromosomal control developed for the active bovine population belonging to the main important cattle and buffalo breeds raised in our country. The goal of our study was to identify chromosomal abnormalities and their effects on the reproductive activity of the carriers, and thus to contribute to improving the genetic health of animals and, consequently, to avoid economic losses at the farm level.

MATERIALS AND METHODS

The material of cytogenetic investigation was represented by 1206 animals: 889 cows from the most common breeds in the country (Romanian Spotted, Romanian Black Spotted, Brown) and 317 buffaloes, from the Romanian buffalo breed. The analysis of chromosomes was carried out by using the short-term blood cell culture. Peripheral blood lymphocytes were cultured for about 72 hours at 38.5°C in Pb-Max (Gibco) complete medium. Two types of cell cultures were performed: without (normal cultures) and with addition of 5-bromodeuxiridine (BrdU) during the last two cell cycles in order to identify the Sister Chromatid Exchanges (SCEs). Slides from both cultures were stained with acridine orange. At least 30 metaphase plates per animal were studied under a fluorescence Nikon microscope, captured with a Photometrics Cool Snap camera, transferred on PC and processed by a specific image software. The cytogenetic

diagnosis was established taking into account the number and structure of chromosomes according to the standard normal karyotype.

RESULTS AND DISCUSSIONS

In cattle, the karyotype control (Table 1) of the 889 females revealed *normal karyotype, 2n=60, XX* for 758 females (Figure 1), 128 cases of *chromosomal instability* and 3 cases of *XX/XY chimerism.*

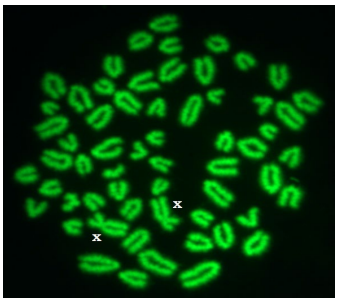

Figure 1. Cattle normal karyotype (own source)

Table 1. The results of cytogenetic investigation in cattle and buffalo breeds

Species/ Breeds	Number of animals	Cytogenetic diagnosis							
		Normal Karyotype		Chromosomal Instability		XX/XY Chimerism		XO Monosomy	
		No	%	No	%	No	%	No	%
Romanian Black Spotted	331	277	83.69	51	15.40	3	0.91		
Romanian Spotted	296	256	86.49	40	13.51				
Brown	170	147	86.47	23	13.53				
Other breeds	92	78	84.78	14	15.22				
Total cattle	**889**	**758**	**85.26**	**128**	**14.40**	**3**	**0.34**		
Buffalo	317	266	83.91	50	15.77			1	0.32
TOTAL	**1206**	**1024**	**84.91**	**178**	**14.76**	**3**	**0.25**	**1**	**0.08**

The chromosomal instability induced by the presence and frequency of many chromatidic breaks on autosomes and heterosomes, lost fragments and gaps (Figure 2) usually affect the integrity of the genetic material with serious consequences for the reproductive capacity of the carriers (Llambi & Postiglioni, 1994; Ducos et al., 2008; Nicolae et al., 2010; Perucatti et al., 2016).

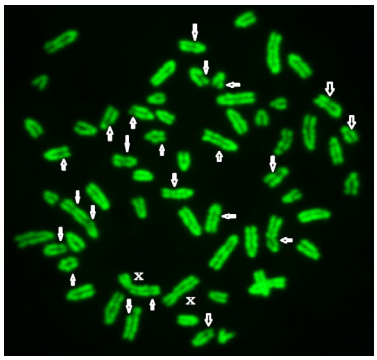

Figure 2. Cattle chromosomal instability (own source)

The ***phenotypic effects of chromosomal instability*** were expressed by reproductive disturbances (repeated inseminations, lack of heat and abortions), 4 cases of *congenital malformations* of the forelimbs (Figure 3), a case of abnormal foetus (*Schistosomus reflexus*) (Figure 6), a case of *posterior limb malformation* (Figure 9) and 3 cases of XX/XY mosaicism.

Congenital malformations of the forelimbs (Figure 3) were identified in 4 cases of *Romanian Black Spotted* calves.



Figure 3. Congenital malformation (own source)

Considering the reproductive disorders reported for their mothers, characterized by abortions and repeated inseminations, taking into account the karyotypic instability, highlighted in both

mothers and calves (Fig. 4), and knowing the possibility of the existence of toxic agents in the environment, which could have effects on the genetic material integrity (Ciotola et al., 2005; Nicolae et al., 2015), we applied the SCEs (Sister Chromatid Exchanges) test to both cows and calves.

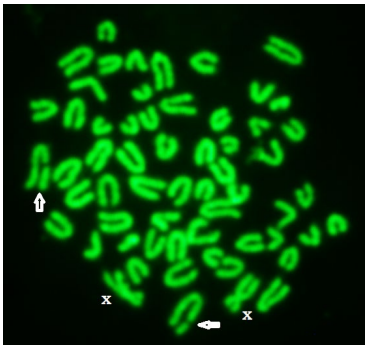

Figure 4. Abnormal configuration in calf with congenital malformation (own source)

Following the SCEs test analysis, it was observed that, if in normal situations the interchromatid exchanges/cell are approx. 6-7 SCEs/cell, in abnormal situations, such as the case of the 4 females and the 4 calves with karyotypic instability, the number of interchromatid exchanges/cell was very high, ranging between 9-13 SCEs/cell (Fig. 5). The presence of 2-3 SCEs on the same chromosome was particularly noted. This could be an indication of the damage to the integrity of the genetic material by environmental pollutants.

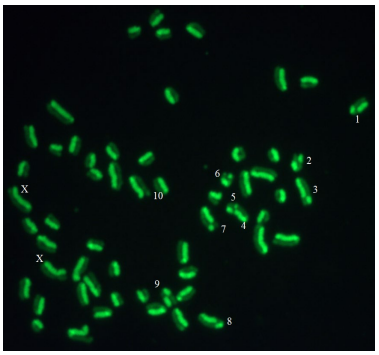

Figure 5. SCEs/ cell indicated by numbers (10) (own source)

Schistosomus reflexus (SR) was *an abnormal foetus,* with a monstrous appearance, dead at birth, described quite rarely in cattle (Azawi et al., 2012; Jana & Jana, 2013; Pandey et al., 2017) but to our knowledge, this is the first case (Figure 6) reported in Romanian Black Spotted dairy cow reared in the farm of the R & D Institute for Bovine Balotesti.

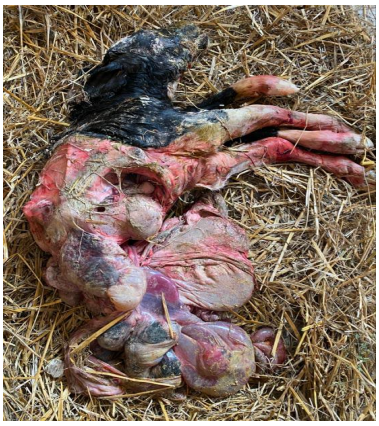

Figure 6. *Schistosomus reflexus (*own source)

Till now this kind of congenital malformation have been associated with genomic instability (Di Berardino et al., 1983; Peretti et al., 2008; Nicolae et al., 2020). To investigate the etiology of this kind of malformation we collected blood samples from both mother and foetus. The chromosomal analysis of the foetus was very difficult because the blood samples contained a lot of clots and we obtained very few good metaphases. After examination it was very clear that the sex of foetus was male. Regarding the mother, the chromosomal configuration was characterized by chromosomal fragility (Figure 7).

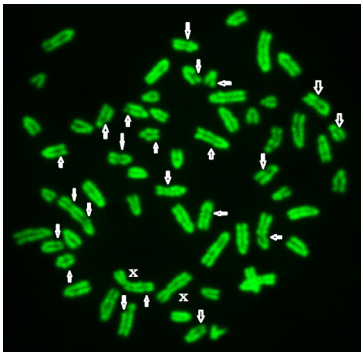

Figure 7. Chromosomal instability of the foetal SR‘s mother (own source)

For this reason, the SCEs test was applied to the mother's metaphases and the observed

interchromatid exchanges (Figure 8) were in a number much higher (9-15 SCEs/cell) than in normal situations in cows (6-7SCEs/cell). Given these results, we can state that the etiology of this congenital malformation could be of teratogenic nature, as other cases have been described before (Nicolae et al., 2020).

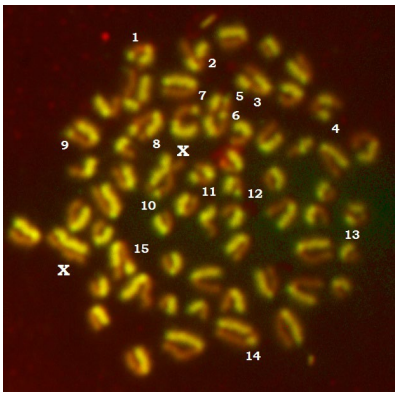

Figure 8. SCEs/ cell indicated by numbers (15) (own source)

Posterior limb malformation (Figure 9), which consists in the lack of the left posterior leg, was identified in a calf from Montbeliarde breed. In this case too, karyotype examination was performed on both the malformed calf and its mother. Following the analysis of metaphases obtained from peripheral blood lymphocyte cultures, chromosomal instability was observed in both the calf's and the mother's cells, but by far, most broken chromosomes were identified in the mother's metaphases, as can be seen in Figure 10.

To gain more information regarding the integrity of the genetic material of the two probands, a detailed analysis has been done by using the SCEs test. The obtained results, revealed a much higher number (Figure 11) of chromatid exchanges (9-15 SCEs/cell) than normal. The presence of double interchromatid exchanges on one, two or even three chromosomes in the same metaphase was very surprising. This is real evidence that the integrity of the genetic material has been very seriously affected. Trying to find out the causes of this malformation, we received information that the mother was treated with antibiotics in the first months of pregnancy.



Figure 9. Posterior limb malformation (own source)

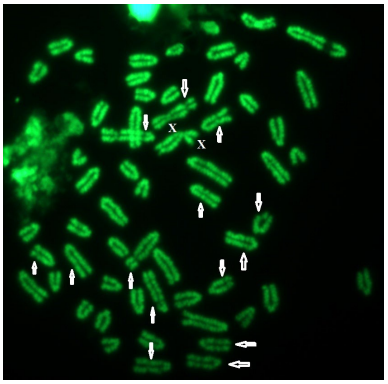

Figure 10. Chromosomal instability of the calf's mother (own source)

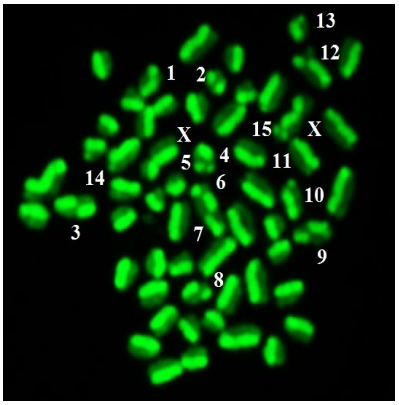

Figure 11. SCEs/cell indicated by numbers (15) (own source)

Also, the presence of aflatoxin on the farm, before and during gestation, has been reported and it is well known that this type of mycotoxin is associated with developmental and

reproductive disorders (Diaz et al., 2004; Akande et al., 2006, Aydin et al., 2008).
All this explains, once again, the teratogenic etiology of this malformation. This is, also, a warning sign for farmers who must be careful about the effects of toxic agents on females during pregnancy, in order to avoid adverse consequences for the offspring and thus, reducing economic losses at farm level.
A special chromosomal configuration ***XX/XY chimerism,*** was found in 3 Romanian Black Spotted females. This is a sex chromosome syndrome which is defined by the abnormal presence of sex chromosomes in the same organism, i.e., cells with XX and cells with XY, in different percentage (Figure 12).

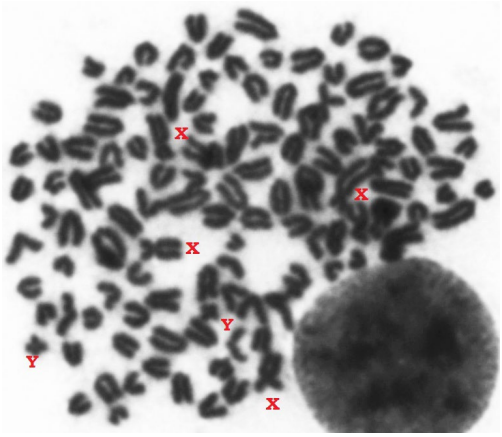

Figure 12. XX/XY Leukocyte chimerism
(one XX cell and two XY cells) (own source)

The presence of leukocyte chimerism in variable proportions, correlated with the degree of reproductive impairment is a reliable and early indicator of the diagnosis of *freemartinism* in the females. For each of our cases, 100 metaphases were examined and it was found that the proportion of XX cells varied between 15 and 35 percent and the proportion of XY cells varied between 65 and 85 percent. These configurations have as effects a serious impairment of the females' genitalia (Fig. 13) and are implicitly associated with sterility of the freemartin females. Taking into account the theoretical premises and the cases described by other authors (Basrur et al., 1970; Nicolae et al., 2001; Iannuzzi et al., 2021), their removal from the herd was recommended.



Figure 13. Abnormal freematine female genitalia (own source)

As well as the artificial inseminations in buffaloes increased, a longstanding interest was dedicated to Romanian buffalo breed too. The chromosomal analysis of 317 buffalo females give us the following results: normal karyotype (2n=50, XX) for 266 females (Figure 14) and chromosomal instability for 50 females.

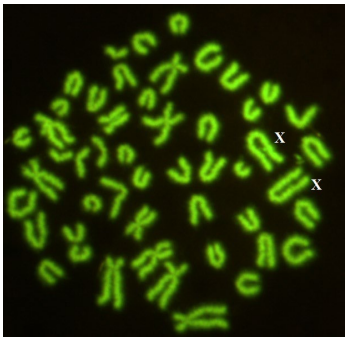

Figure 14. Buffalo normal karyotype (own source)

As can be seen in Figure 15, the damage to the chromosome structure in buffalo metaphases is evident: very clear breaks and lost fragments.

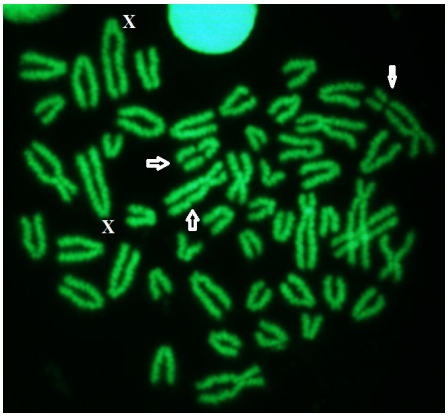

Figure 15. Buffalo chromosomal instability
(own source)

In order to investigate and identify the causes that led to this situation, the SCEs test was applied, and we observed a very high number of interchromatid exchanges/cell, ranging between 10-14 SCEs/cell (Figure 16 A, B.), compared to the number found in normal situations in buffalo (7-8 SCEs/cell). It was also remarkable the double number of SCEs on the chromosomes of the same cell. This detail draws attention to the possible damage generated by environmental pollution.

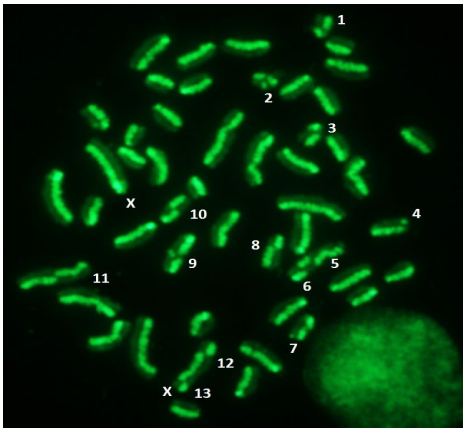

Figure 16 A. Buffalo metaphase with 13 SCEs/cell
(own source)

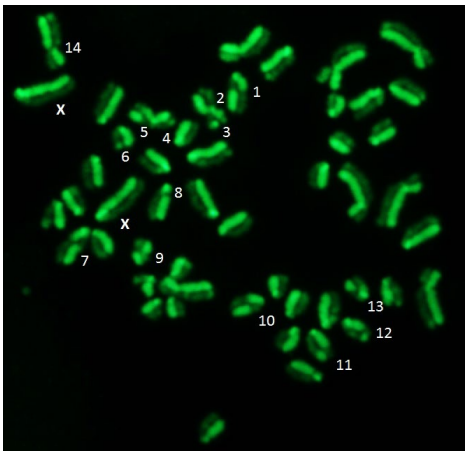

Figure 16 B. Buffalo metaphases with 14 SCEs/cell
(own source)

We also identified an aneuploid-heteroploidy of sex chromosomes **(2n=49, X0**), well known as ***X-monosomy*** or ***Turner's syndrome*****,** in a buffalo female with prominent withers and tight pelvis. Till now, only a few cases were described (Yadav et al., 1990; Prakash et al., 1992; Iannuzzi et al., 2000, Iannuzzi et al., 2021), and all were characterized by different alterations of internal genitalia and sterility.
In our case (Figure 17) in all metaphases we observed only one X chromosome and the analysis of the female's reproductive activity pointed out reproduction disorders and infertility.

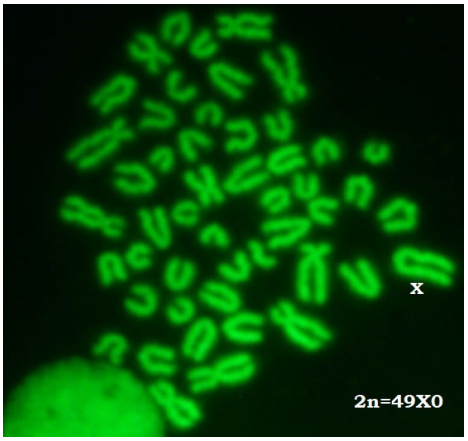

Figure 17. Buffalo X-monosomy
(own source)

For all investigated animals, chromosomal analysis bulletins have been released and appropriate prophylaxis measures have been recommended.

CONCLUSIONS

Cytogenetic investigation of 1206 females from 2 species (cattle and buffalo) of different Romanian breeds revealed *abnormal karyotype for 131 cattle and 51 buffaloes.* The large number of animals, identified with karyotypic instability, the high percentage both in cattle (14.40%) and buffalo (15.77%) as well as the effects recorded on their reproductive capacity, demonstrated once again that the damage of genetic material integrity can induce developmental and reproduction disorders of carriers. All of these, highlights that the only possibility of intervention in maintaining the genetic health of the herd is to identify abnormal genotypes and eliminate them from the population, thus avoiding the risk of dissemination in offspring. *In all cases, chromosomal analysis bulletins have been released and appropriate prophylaxis measures have been recommended*.

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